PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 August 2000 (02.08.00)	
International application No. PCT/EP99/10297	**Applicant's or agent's file reference** ML/B45168
International filing date (day/month/year) 21 December 1999 (21.12.99)	**Priority date** (day/month/year) 21 December 1998 (21.12.98)
Applicant BOLLEN, Alex et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

24 June 2000 (24.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pascal Piriou

Telephone No.: (41-22) 338.83.38

We claim:

1. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

2. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

3. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

4. An isolated polypeptide comprising a fragment of the polypeptide as claimed in any one of claims 1 to 3.

5. The polypeptide of claim 4, wherein the fragment is immunogenic.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over the entire length of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over the entire length of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

13. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 - 12.

14. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence that has at least 75% identity to the amino acid sequence selected from the group consisting of:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

15. A process for producing a polypeptide comprising an amino acid sequence that has at least 75% identity to the amino acid sequence selected from the group consisting of SEQ ID NO: 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 comprising culturing a host cell of claim 14 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

16. A process for expressing a polynucleotide of any one of claims 6 - 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

17. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

18. The vaccine composition of claim 17, wherein the polypeptide has an amino acid sequence selected from the group consisting of: SEQ ID NO:42, 46, 48, 50, 52, 54, 56, 58, 60 and 62.

19. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically acceptable carrier.

20. The vaccine composition according to any one of claims 17-19, wherein said composition comprises at least one other *Bordetella pertussis* antigen.

21. An antibody immunospecific for the polypeptide or fragment as claimed in any one of claims 1 to 5.

22. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 5, or an antibody that is immunospecific

for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

23. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 – 5 in the preparation of a medicament for use in generating an immune response in an animal.

24. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 - 12 in the preparation of a medicament for use in generating an immune response in an animal.

25. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claims 1 – 5 and a suitable pharmaceutical carrier.

26. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 3-18 or a polypeptide of claim 19.

27. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;

sequencing the *lcrD*-like gene;

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

if a virulence-associated member, sequencing the entire pathogenicity island, and

identifying genes within this sequence.

28. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

ATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
ML/B45168		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/10297	21/12/1999	21/12/1998
International Patent Classification (IPC) or national classification and IPC C07K14/235		
Applicant UNIVERSITE LIBRE DE BRUXELLES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24/06/2000	09.04.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Roscoe, R Telephone No. +49 89 2399 2554

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/10297

I. Basis f th r p rt

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-46 as originally filed

Claims, No.:

1-29 as received on 23/03/2001 with letter of 23/03/2001

Drawings, sheets:

1/28-28/28 as originally filed

Sequence listing part of the description, pages:

1-84 (numbered 47-130 by applicant), as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

☐ copy of the earlier application whose priority has been claimed.

☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. (1-26)part, 27-28.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. (1-26)part, 27-28.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. (1-26)part.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 2, 3, 10, 11, 19
	No:	Claims 1, 4-9, 12-18, 20-27

Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-27
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-27

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The documents mentioned in the present written opinion / International Preliminary Examination Report are numbered as in the search report, i.e. D1 corresponds to the first document of the search report etc.

II. Priority

bopN and orf6 are both already identified in the priority document. Priority seems to be valid for the present claims insofar as they are subject to examination. Hence, documents D4 to D6 are not considered relevant to the claims presently under examination.

III. No Opinion

No opinion can be expressed for unsearched subject-matter. Hence, this examination is conducted only on claims 1-26, insofar as they relate to sequence Nos 41/42 and 53/54.

IV. Lack of Unity

The IPEA has decided not to request applicant to pay additional fees for the Examination of both invention groups 1 and 7, as defined in the Search Report.

Nevertheless, applicant should note that the IPEA agrees entirely with the grouping of the inventions and the reasoning underlying these objections, i.e. that given that the pathogenicity Island was already known (D1), and a gene from it (YscN homolog), further genes from the island cannot be considered unified by their location therein. Further, the genes have no specific function attributed to them and thus cannot be stated to solve any specific problem.

V. Reasoned statement on Novelty, Inventive Step and Industrial Applicability

- Novelty (Art.33(2) PCT)

None of the cited prior art documents disclose the bopN or ORF6 genes / proteins and related matter.

Claims 1, 4-9, 12-18 all are not novel due to a lack of clarity as documented below (since most known proteins/DNAs and their related applications can fall within the scope of the present claims). Since there is no cited prior art providing similar sequences to the specific sequence IDs upon which the claims are founded, it should easily be possible to overcome this problem without an undue restriction in scope.

Claims 20-27 are also refering to inadequate protein definitions and are thus also effectively not novel over the routine applications of any putative Bordetella pertussis protein antigen.

Further, it is noted with respect to claims 22 and 26 that claims 1, 2, 4 and 5 are open-ended (i.e. only e.g. 75% of sequence defined, other 25% can be absolutely anything) - hence antibody binding to any protein can fall under scope of claim 21. Antibodies must be limited to those binding an exact sequence.

For the benefit of the applicant it is again stated that were the present claims limited to the exact sequences of Seq.ID Nos 41/42 or 53/54 and to sequences which have 75% identity to Seq.ID Nos 42 or 54 over the entire length of these defined sequences then all of the presently examined claims apart from 22 and 26 could be considered novel.

- **Inventive Step (Art.33(3) PCT)**

Applicant provides two genes which were found in the vicinity of a cluster of B. pertussis type III secretion apparatus genes. The existence of such genes in B. pertussis is demonstrated in D1, which also shows how these genes are physically clustered in B.bronchosepta (see Fig.2). None of these genes are type III effector genes (or class II genes, using applicants terminology). Hence, D1 does not prove that the effector genes are adjoining the secretory genes in B.pertussis. The presence of type III effector genes is only functionally demonstrated by switching on/off the regulon and looking for changes in protein secretion or virulence characteristics.

D2 discloses known type III secretion systems and genes comprised therein.

Applicant identifies class II ORFs based on their proximity to class I ORFs. This approach is basically only made credible by the prior art which already shows that type III genes tend to be clustered in pathogenicity islands (applicant admits this, p.36, l.4-5). Applicant has generally no other basis for claiming that these genes are effector genes (only in case of bopN, a similarity to YopN is indicative of the gene belonging to class II). This fact is however precisely why inventive step cannot be acknowledged. Like many other virulence systems, the type III secretion system is likely to have been disseminated from one species to another by co-transfer of a cluster of secretory and effector genes. Had only one class of genes been transfered (e.g. conjugatively) then it would have had no function (since specific sequence on effector proteins interacts with specific secretion machinery) and would have been rapidly removed from the bacterial population by negative selection. A skilled person would undoubtedly assume that the locus was functional (i.e. depending on culture conditions), and irrespective of this would be interested in studying it further, as indicated in D1. The pYV plasmid of Yersinia demonstrates clustering of secretory / effector proteins and may be a good example of a dissemination vehicle for the gene cluster. SPI1 and SPI2 in Salmonella and the LEE locus of enteropathogenic E. coli (EPEC) provide further examples of type III secretory loci comprising both secretory and effector genes. None of the prior art type III systems identified so far appears to involve a discrete secretory locus and a seperate locus or disseminated effector genes.

The problem solved by applicant is to find class II genes of a type III secretory system in B. pertussis. Applicants solution is (i) bopN and (ii) ORF6. No specific function is provided so no more specific problem can be considered to have been solved. In view of the prior art, it is obvious that class II genes are expected in the vicinity of class I genes and hence it is obvious to sequence DNA adjoining class I genes and to designate the adjoining genes as putative effector (class II) genes.

Claims considered devoid of inventive subject-matter since either directly refer to non-inventive DNA / protein or to obvious derivatives and uses thereof which are trivial known uses in the context of class II effector genes (i.e. vaccine / probe).

- **Industrial Applicability (Art.33(4) PCT)**

Since the function of ORF6 is not known and it is not known if it is a suitable vaccine target, all matter relating thereto is considered to lack industrial applicability (i.e. claims 1-26).

VI. Certain documents

In accordance with Rule 70.10, PCT, applicants attention is drawn to the following document(s):

WO-A-99/59630 (Publication date, 25.11.99; Priority date, 15.5.98; Filing date, 14.5.99)

VIII. Certain observations

- Clarity (Art.6 PCT)

Amended claims 1, 4, 6, 7(2 aspects), 8(2 aspects), 9, 12, 14, 15 - all still fail to define lengths over which identity is found, length of complementary sequence, or size of fragment. This problem must be rectified in all of these claims. The insertion of "over its entire length" is not helpful here because "its" clearly refers to the claimed sequence (not the Seq ID sequences). Thus, if the claimed sequence is 2 aa long and identical to 2 consecutive amino acids of one of the sequence ID Nos it will be even 100% identical over its full length. Hence, in principle none of these claims are novel and the clarity issue will have to be appropriately addressed.

Claim 12 - stringent conditions need to be defined in the claims

Claim 15 - the subcellular fraction need not have any of protein(s) of invention. Same applies to membrane

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

To:
SMITHKLINE BEECHAM
Corporate Intellectual Property
Attn. Tyrrell, William A.R.
Two New Horizons Court
Brentford
Middlesex TW8 9EP
UNITED KINGDOM

Applicant's or agent's file reference ML/B45168	Date of mailing (day/month/year) 24/08/2000
International application No. PCT/EP 99/10297	**PAYMENT DUE** within 30 ~~months~~/days from the above date of mailing
	International filing date (day/month/year) 21/12/1999

Applicant

UNIVERSITE LIBRE DE BRUXELLES et al.

1. This International Searching Authority

(i) considers that there are 17 *(number of)* inventions claimed in the international application covered by the claims indicated ~~below~~/on the extra sheet:

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated ~~below~~/on the extra sheet:

(ii) [X] has carried out a partial international search (see Annex) [ ] will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:

1-26, all partly

(iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby **invited**, within the time limit indicated above, to pay the amount indicated below:

EUR 945,00	x	16	=	EUR 15.120,00
Fee per additional invention		number of additional inventions		total amount of additional fees

Or, _____ x _____ = _____

The applicant is informed that, according to Rule 40.2(c), **the payment of any additional fee may be made under protest,** i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

3. [ ] Claim(s) Nos. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chantal Meyer

Form PCT/ISA/206 (July 1992)

Annex to Form PCT/ISA/206
COMMUNICATION RELATING TO THE RESULTS OF THE PARTIAL INTERNATIONAL SEARCH

International Application No
EP 99/10297

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claims Nos.:
see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.

3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.

4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YUK M H ET AL: "THE BVGAS VIRULENCE CONTROL SYSTEM REGULATES TYPE III SECRETION IN BORDETELLA BRONCHISEPTICA" MOLECULAR MICROBIOLOGY,GB,BLACKWELL SCIENTIFIC, OXFORD, vol. 28, no. 5, 1998, pages 945-959, XP002922650 ISSN: 0950-382X page 946 page 952, column 2, last paragraph ---	1-26
A	LEE C A: "TYPE III SECRETION SYSTEMS: MACHINES TO DELIVER BACTERIAL PROTEINS INTO EUKARYOTIC CELLS?" TRENDS IN MICROBIOLOGY,GB,ELSEVIER SCIENCE LTD., KIDLINGTON, vol. 5, no. 4, April 1997 (1997-04), pages 148-156, XP002922657 ISSN: 0966-842X the whole document ---	1-26
P,X	WO 99 59630 A (UNIVERSITY OF CALIFORNIA) 25 November 1999 (1999-11-25) the whole document, especially figures 18 and 21 and claims 22 and 25 ---	1-26

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

International Application No
[redacted]EP 99/10297

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	FAUCONNIER A ET AL.: "Characterization of a locus encoding a type III secretion system in Bordetella pertussis" ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY., vol. 99, 30 May 1999 (1999-05-30), page 31 XP000929802 WASHINGTON US ISSN: 0067-2777 the whole document ---	1-26
P,X	KERR J.R. ET AL.: "The Bpel locus encodes type III secretion machinery in Bordetella pertussis" MICROBIAL PATHOGENESIS, vol. 27, no. 6, December 1999 (1999-12), pages 349-367, XP000925861 LONDON GB the whole document ---	1-26
T	GALAN J E ET AL: "TYPE III SECRETION MACHINES: BACTERIAL DEVICES FOR PROTEIN DELIVERYINTO HOST CELLS" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 284, 21 May 1999 (1999-05-21), pages 1322-1328, XP002922658 ISSN: 0036-8075 the whole document -----	1-26

1

INVITATION TO PAY ADDITIONAL FEES

International application No.
PCT/EP 99/10297

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26, all partly

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 42;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.41; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

2. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 44;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.43; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

3. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 46;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.45; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

4. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 48;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.47; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said

polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

5. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 50;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.49; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

6. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 52;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.51; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

7. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 54;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.53; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

8. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 56;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.55; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said

polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

9. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 58;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.57; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

10. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 60;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.59; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

11. Claims: 1-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 62;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.61; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

12. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 64;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.63; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said

polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

13. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 66;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.65; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

14. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No.68;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.67; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

15. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 70;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.69; an expression vector comprising said polynucleotide; a host cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

16. Claims: 1-17, 19-26, all partially

An isolated polypeptide having at least 75% identity to the amino acid sequence of SEQ.ID.No. 72;an isolated polynucleotide encoding said polypeptide and having at least 75% identity to the nucleotide sequence of SEQ.ID.No.71; an expression vector comprising said polynucleotide; a host

cell comprising said vector; a process for producing said polypeptides c.q. polynucleotides; a vaccine comprising said polypeptides c.q. polynucleotides; an antibody against said polypeptide and the diagnostic and therapeutic use of said polypeptides, polynucleotides and antibodies.

17. Claims: 27, 28

A method of identifying virulence genes from a pathogenicity island containing a type III secretion system and a method of determining whether a bacterial strain harbours a type III secretion system.

1. The problem underlying the present application is the identification of class II virulence effector proteins from pathogenic bacteria. Inventions 1 to 16 identified above relate to different virulence proteins and invention 17 relates to a method of their identification. The common concept in the sense of Rule 13.1 PCT being that all the virulence proteins have been derived from a pathogenicity island containing a Type III secretion system, present in the Bordetella pertussis genome.

2. However, the above-mentioned pathogenecity island has been described already in the prior art. Yuk M.H. et al (Molecular Microbiology, vol. 28, no.5, may 1998, pages 945-959) have identified a locus encoding the homolog of the YscN gene of Yersinia, which is part of the type III secretion apparatus, in Bordetella bronchiseptica (page 946), as well as flanking sequences showing open reading frames (ORF's) similar to other members of the type III secretion system. They have also shown that Bordetella pertussis also contains (and expresses)(see page 952, column 2, last paragraph)this locus. That the locus described by Yuk et al is the same as the locus identified in the present application can be inferred from the fact that both contain the YscN homolog (see Table, SEQ.ID.No.:21).

3. Thus, in view of the above-mentioned prior art, the common concept is not new and the problems underlying the present application can be defined as:
i) providing further virulent polypeptides derived from the above-mentioned pathogenicity island, inventions 1-16 being separate solutions to this problem and
ii) providing a further method for their identification, invention 17 being the solution to this problem.

4. In summary, due to the fact that proteins derived from the pathogenicity island containing a Type III secretion system present in the Bordetella pertussis genome have already been disclosed, and due to the fact that essential differences exist in the sequences of the polypeptides and polynucleotides claimed in inventions 1 to 16 respectively and due to the fact that no other technical features can be distinguished which, in the light of the prior art could be regarded as special technical features in the sense of Rule 13.2 PCT, the ISA is of

the opinion that there is no single inventive concept underlying the inventions of the present application in the sense of Rule 13.1 PCT.
Consequently there is lack of unity and the different inventions, not belonging to a common inventive concept, are formulated as the different subjects on the communication pursuant to Art. 17(3)(a)PCT.

5. It should be noted that for regrouping the different subclaims, the ISA has taking into account the balance between necessary search efforts and the levying of additional fees.

Patent Family Annex

Information on patent family members

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(54) Title: TYPE III SECRETION SYSTEM ANTIGENS FROM BORDETELLA PERTUSSIS

(57) Abstract: This invention relates to a general method for detecting pathogenic strains of bacteria which harbour a type III secretion system. More particularly, this invention relates to the methods as applied to the pathogen *Bordetella pertussis*. Furthermore, the invention relates to newly identified polynucleotides within these regions, virulent polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly the polynucleotides and polypeptides of the present invention relate to the virulent effector proteins associated with the type III secretion system of *Bordetella pertussis*, which are particularly suitable for vaccine purposes.